(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 June 2001 (14.06.2001)

PCT

(10) International Publication Number
WO 01/42192 A2

(51) International Patent Classification[4]: C07C 233/00

(21) International Application Number: PCT/EP00/12226

(22) International Filing Date: 5 December 2000 (05.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/455,670 7 December 1999 (07.12.1999) US

(71) Applicant *(for all designated States except AT, US)*: NOVARTIS AG [CH/CH]; Schwarzwaldallee 215, CH-4058 Basel (CH).

(71) Applicant *(for AT only)*: NOVARTIS-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H [AT/AT]; Brunner Strasse 59, A-1230 Vienna (AT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: WATTANASIN, Sompong [TH/US]; 11 Divito Trail, Hopatcong, NJ 07832 (US). WEIDMANN, Beat [CH/US]; 20 Van Doren Avenue, Chatham, NJ 07928 (US).

(74) Agent: BECKER, Konrad; Novartis AG, Corporate Intellectual Property, Patent & Trademark Department, CH-4002 Basel (CH).

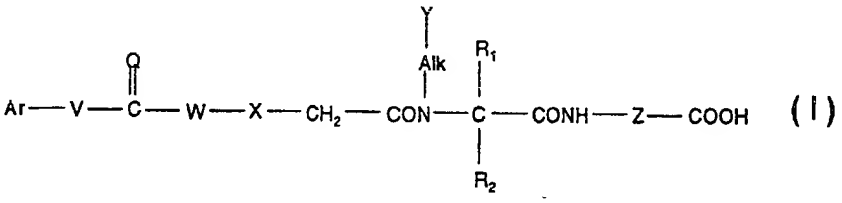
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

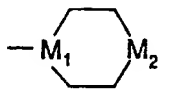
Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: VLA-4 INTEGRIN ANTAGONISTS



(I)



(II)

(57) Abstract: Compounds of formula (I) wherein Ar is carbocyclic or heterocyclic aryl, or biaryl; Q is O, S or N-C≡N; X is arylene; V is NH, O, NHOH, CH_2 or a direct bond; W is NH, O, NHOH, CH_2 or a direct bond; Alk is C_2-C_7-alkylene or C_2-C_7-alkylene interrupted by O, S, SO, SO_2 or NR_3; Y is amino, acylamino, mono- or di- (lower alkyl, aryl or aralkyl)-amino, pyrrolidino, perhydroazepino or a group of the formula (II) in which M_1 is N; and M_2 is CH_2, O, NR_3, S, SO or SO_2; R_1, R_2 and R_3 are independently hydrogen, lower alkyl, lower alkenyl, cycloalkyl, aryl, cycloalkyl-lower alkyl, aryl-lower alkyl or aryl-lower alkenyl; Z is lower alkylene or lower alkylene substituted by one or more of lower alkyl, lower alkenyl, cycloalkyl, aryl, cycloalkyl-lower alkyl, aryl-lower alkyl or aryl-lower alkenyl; or Z is lower alkylene interrupted by O, S, SO, SO_2 or NR_3; pharmaceutically acceptable esters and amides thereof; and pharmaceutically acceptable salts thereof, which are useful as VLA-4 integrin antagonists.

WO 01/42192 A2

AQ2

VLA-4 INTEGRIN ANTAGONISTS

Cell adhesion (i.e., a process by which cells associate with each other, migrate towards a specific target, or localize within the extracellular matrix) underlies many biological phenomena. Cell adhesion causes adhesion of hemoatopoietic to endothelial cells and the subsequent migration of those hemopoietic cells out of blood vessels and to the site of injury, thus playing a role in mammalian pathologies such as inflammation and immune reactions.

Various cell-surface macromolecules (known as cell adhesion receptors) mediate cell-cell and cell-matrix interactions. For example, the integrins are the key mediators in adhesive interactions between hematopoietic and other cells. Integrins are non-covalent heterodimeric complexes consisting of two subunits, α and β. Depending on the type of its α and β subunit components, each integrin molecule is categorized into its own subfamily. There are at least 12 different α subunits (α1-α6, α-L, α-M, α-X, α-IIB, α-V, and α-E) and at least 9 different β subunits (β1-β9).

The integrin VLA-4 (very late antigen-4), also known as α4β1 integrin or CD49d/CD29, is a leukocyte cell surface receptor that participates in a variety of cell-cell and cell-matrix adhesions. It is a receptor for both the cytokine-inducible endothelial cell surface protein, vascular cell adhesion molecule-1 (VCAM-1), and the extracellular matrix protein fibronectin (FN). Anti-VLA-4 monoclonal antibodies (mAb's) inhibit VLA-4-dependent adhesive interactions both in vitro and in vivo. The inhibition of VLA-4-dependent cell adhesion may prevent or inhibit several inflammatory and autoimmune pathologies.

VLA-4 Antagonists are disclosed e.g. in International Application WO 96/22966.

SUMMARY OF THE INVENTION

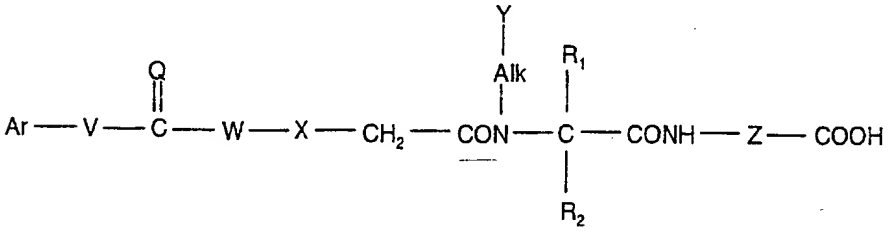
The invention relates to the compounds as defined herein which are potent VLA-4 antagonists and methods for preparation thereof, pharmaceutical compositions comprising said compounds, and methods of inhibiting VLA-4 and of treating,

preventing or suppressing conditions in mammals which are responsive to VLA-4 inhibition using said compounds or pharmaceutical compositions comprising said compounds.

The compounds of the invention are useful to inhibit, prevent and suppress VLA-4 mediated cell adhesions. They are thus useful for the treatment of VLA-4 mediated conditions, particularly inflammation, autoimmune diseases, and immune reactions to e.g. organ transplantation. Conditions in which VLA-4 is implicated include rheumatoid arthritis, respiratory diseases such as asthma, multiple sclerosis, and complications of organ transplantation, e.g. of heart, lung, pancreas (islet) transplantation.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to the novel compounds of formula I



(I)

wherein

Ar is carbocyclic or heterocyclic aryl, or biaryl;

Q is O, S or N-C≡N;

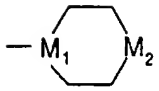
X is arylene;

V is NH, O, NHOH, CH_2 or a direct bond;

W is NH, O, NHOH, CH_2 or a direct bond;

Alk is C_2-C_7-alkylene or C_2-C_7-alkylene interrupted by O, S, SO, SO_2 or NR_3;

Y is amino, acylamino, mono- or di- (lower alkyl, aryl or aralkyl)-amino, pyrrolidino, perhydroazepino or a group of the formula

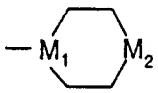


in which M_1 is N; and M_2 is CH_2, O, NR_3, S, SO or SO_2;
R_1, R_2 and R_3 are independently hydrogen, lower alkyl, lower alkenyl, cycloalkyl, aryl, cycloalkyl-lower alkyl, aryl-lower alkyl or aryl-lower alkenyl;
Z is lower alkylene or lower alkylene substituted by one or more of lower alkyl, lower alkenyl, cycloalkyl, aryl, cycloalkyl-lower alkyl, aryl-lower alkyl or aryl-lower alkenyl; or Z is lower alkylene interrupted by O, S, SO, SO_2 or NR_3;
pharmaceutically acceptable esters and amides thereof; and pharmaceutically acceptable salts thereof.

Compounds of the invention may possess one or more asymmetric carbon atoms and can exist as diastereomers, racemates and the enantiomers thereof, all of which are within the purview of the invention.

Particular embodiments of the invention relate to the compounds wherein V and W are NH or NHOH; or wherein V is CH_2 and W is NH; or wherein V is a direct bond and W is NH; or wherein V is NH and W is CH_2; also to the above compounds wherein Q is O, S or N-C≡N.

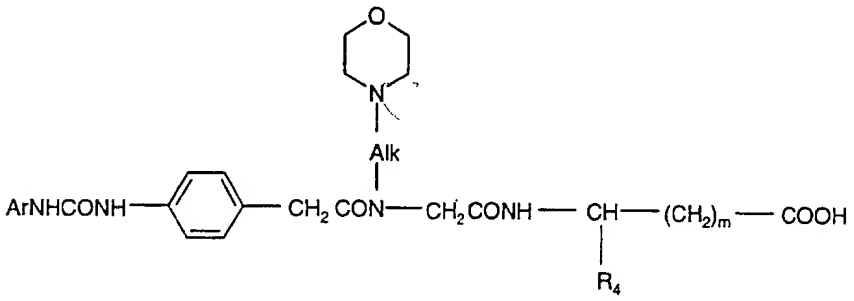
Preferred are the compounds of formula I wherein V and W are NH; Q is O; X is phenylene; Ar is carbocyclic or heterocyclic aryl; Alk is C_2-C_4-alkylene; R_1, R_2 and R_3 are hydrogen or lower alkyl; Y is a group of the formula



in which M_1 is N and M_2 is CH_2, O or S; Z is C_2-C_5- straight chain alkylene optionally substituted by lower alkyl, lower alkenyl, carbocyclic aryl or heterocyclic aryl; or Z is C_2-

C_5- straight chain alkylene interrupted by O, S, SO or SO_2; pharmaceutically acceptable esters and amides thereof; and pharmaceutically acceptable salts thereof.

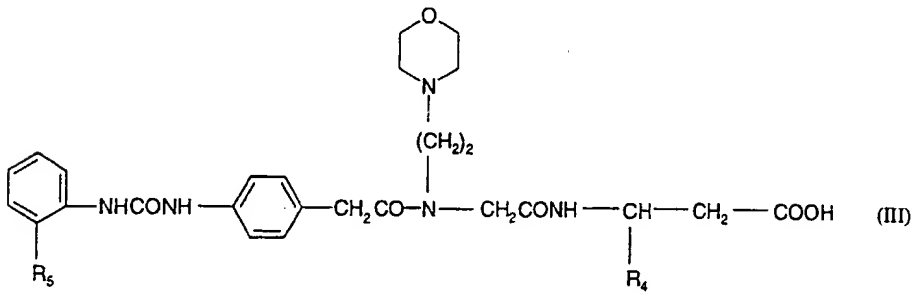
Further preferred are the compounds of formula II



(II)

wherein Ar is monocarbocyclic aryl; Alk is C_2-C_4-alkylene;
R_4 is lower alkyl, lower alkenyl, or monocarbocyclic aryl; m is 1 or 2; pharmaceutically acceptable esters thereof; and pharmaceutically acceptable salts thereof.

Most preferred are the compounds of formula III



(III)

wherein R_4 is phenyl or phenyl substituted by one to three of C_1-C_4-alkoxy, chloro, fluoro or C_1-C_4-alkyl; and R_5 is C_1-C_4-alkoxy, chloro, fluoro, or C_1-C_4-alkyl; pharmaceutically acceptable esters thereof; and pharmaceutically acceptable salts thereof.

Unless otherwise indicated, the general definitions used herein have the following meaning within the scope of the present invention.

Aryl represents carbocyclic or heterocyclic aryl, either monocyclic or bicyclic.

Arylene is an aryl linking group in which aryl is heterocyclic or carbocyclic aryl, preferably monocyclic carbocyclic aryl.

A carbocyclic aryl linking group (as for X in formula I) is for instance optionally substituted phenylene to which the two adjacent groups are attached either ortho, meta or para, preferably para, to each other.

Monocyclic carbocyclic aryl represents optionally substituted phenyl, being preferably phenyl or phenyl substituted by one to three substituents, such being advantageously lower alkyl, hydroxy, lower alkoxy, acyloxy, halogen, cyano, trifluoromethyl, carbocyclic aryloxy or carbocyclic aryl-lower alkoxy.

Bicyclic carbocyclic aryl represents 1- or 2-naphthyl or 1- or 2-naphthyl substituted by, e.g., lower alkyl, lower alkoxy or halogen.

Monocyclic heterocyclic aryl represents optionally substituted thienyl, furanyl, pyridyl, pyrrolyl, thiazolyl, pyrazinyl, pyridazinyl or pyrazolyl, preferably optionally substituted thiazolyl, thienyl, furanyl or pyridyl.

Optionally substituted furanyl represents 2- or 3-furanyl preferably substituted by lower alkyl.

Optionally substituted pyridyl represents 2-, 3- or 4-pyridyl or 2-, 3- or 4-pyridyl preferably substituted by lower alkyl, halogen or cyano.

Optionally substituted thienyl represents 2- or 3-thienyl or 2- or 3-thienyl preferably substituted by lower alkyl.

Optionally substituted thiazolyl represents, e.g., 4-thiazolyl, or 4-thiazolyl substituted by lower alkyl.

Bicyclic heterocyclic aryl represents e.g. quinolinyl, isoquinolinyl, indolyl or benzothiazolyl optionally substituted by hydroxy, lower alkyl, lower alkoxy or halogen.

Aryl as in aryl-lower alkyl is preferably phenyl or phenyl substituted by one or two of lower alkyl, lower alkoxy, hydroxy, acyloxy, halogen, trifluoromethyl or cyano; also, optionally substituted naphthyl.

Aryl-lower alkyl is advantageously benzyl or 1- or 2-phenethyl optionally substituted on phenyl by one or two of lower alkyl, lower alkoxy, hydroxy, lower alkanoyloxy, halogen, cyano or trifluoromethyl.

Biaryl represents phenyl substituted by carbocyclic aryl or heterocyclic aryl as defined herein, ortho, meta or para to the point of attachment of the phenyl ring, advantageously para, such as 4-biphenyl.

The term "lower" referred to herein in connection with organic radicals or compounds respectively defines such with up to and including 7, preferably up and including 4 and advantageously one or two carbon atoms. Such may be straight chain or branched.

A lower alkyl group preferably contains 1-4 carbon atoms and represents for example, ethyl, propyl, butyl or advantageously methyl.

A lower alkylene group preferably contains 1-4 carbon atoms, and represents, for example, ethylene, propylene and the like.

A lower alkenyl group preferably contains 2-4 carbon atoms, and represents, for example, allyl.
Cycloalkyl represents preferably cyclopentyl, cyclohexyl or cycloheptyl.

A lower alkoxy group preferably contains 1-4 carbon atoms and represents for example, methoxy, propoxy, isoproproxy or advantageously ethoxy.

Halogen (halo) preferably represents fluoro or chloro, but may also be bromo or iodo.

Acyl is derived from a carboxylic acid or carbonic acid and represents preferably optionally substituted lower alkanoyl, aroyl , lower alkoxycarbonyl or aryl-lower alkoxycarbonyl, advantageously aroyl.

Lower alkanoyl is preferably acetyl, propionyl, butyryl, or pivaloyl.

Optionally substituted lower alkanoyl for example represents lower alkanoyl or lower alkanoyl substituted e.g. by lower alkoxycarbonyl, lower alkanoyloxy, lower alkanoylthio, lower alkoxy, or by lower alkylthio.

Aroyl is preferably monocyclic carbocyclic or monocyclic heterocyclic aroyl.

Monocyclic carbocyclic aroyl is preferably benzoyl or benzoyl substituted by lower alkyl, lower alkoxy, halogen or trifluoromethyl.

Monocyclic heterocyclic aroyl is preferably pyridylcarbonyl or thienylcarbonyl.

Acyloxy is preferably optionally substituted lower alkanoyloxy, lower alkoxycarbonyloxy, monocyclic carbocyclic aroyloxy or monocyclic heterocyclic aroyloxy.

Aryl-lower alkoxycarbonyl is preferably monocyclic carbocyclic-lower alkoxycarbonyl, advantageously benzyloxycarbonyl.

Pharmaceutically acceptable esters are preferably prodrug ester derivatives, such being convertible by solvolysis or under physiological conditions to the free carboxyclic acids of formula I.

Pharmaceutically acceptable esters are preferably prodrug esters, e.g. lower alkyl esters, cycloalkyl esters, lower alkenyl esters, benzyl esters, mono or disubstituted lower alkyl esters, e.g. the ω-(amino, mono- or di- lower alkylamino, carboxy, lower alkoxycarbonyl)-lower alkyl esters, the α-(lower alkanoyloxy, lower alkoxycarbonyl or di-lower alkylaminocarbonyl)-iower alkyl esters, such as the pivaloyloxy-methyl ester, and the like conventionally used in the art.

Pharmaceutically acceptable amides are e.g. primary, secondary and tertiary amides, i.e. the unsubstituted, the N-mono-lower alkyl or N,N-di-lower alkylamides or amides of cyclic amines, e.g. of piperidine, pyrrolidine, morpholine or optionally substituted piperazine.

Pharmaceutically acceptable salts of the acids of the invention are salts derived from pharmaceutically acceptable bases, e.g. alkali metal salts (e.g. sodium, potassium salts), alkaline earth metal salts (e.g. magnesium, calcium salts) amine salts (e.g. ethanolamine, diethanolamine, lysine and tromethamine salts) and the like conventionally used in the art.
When a compound of the present invention contains a basic group, salts may also be prepared from pharmaceutically acceptable non-toxic acids, such as mineral acids, e.g. hydrochloric, sulfuric or phosphoric acid, or organic acids, for example aliphatic or aromatic carboxylic or sulfonic acids, e.g. succinic, lactic, malic, citric, maleic, fumaric, methanesulfonic, acetic acid and the like.

The compounds of the invention are particularly useful in mammals as VLA-4 antagonists, for inhibiting VLA-4 associated cell adhesions and for treating, ameliorating or preventing conditions in mammals in which VLA-4 is implicated, e.g. immune, autoimmune disorders and inflammatory conditions. Such include respiratory disorders (e.g. asthma), arthritis (e.g. rheumatoid arthritis), psoriasis, transplantation rejection, multiple sclerosis, type I diabetes, inflammatory bowel disease, sickle cell anemia, Crohn's disease, uveitis, systemic lupus erythematosus, myasthenia gravis, gout, and the like.

As to the respiratory diseases, the compounds of the invention are useful as agents for the symptomatic or prophylactic treatment of inflammatory airways diseases. Such diseases include asthma of whatever type or genesis including both intrinsic and, especially, extrinsic asthma. They are useful for the treatment of allergic asthma, whether atopic (i.e. IgE-mediated) or non-atopic, as well as bronchitic asthma, exercise-induced asthma, occupational asthma, asthma induced following bacterial infection and other non-allergic asthmas. Treatment of asthma is also to be understood as embracing treatment of patients of less than 4 or 5 years of age exhibiting wheezing symptoms, particularly at night and diagnosed or diagnosable as "wheezy infants".

Prophylactic efficacy in the treatment of asthma may be manifested by reduced frequency or reduced severity of symptomatic attack. It may be further evidenced by reduced requirement for symptomatic therapy, i.e. therapy for, or intended to restrict or abort, symptomatic attack when it occurs, for example for anti-inflammatory therapy using a corticosteroid.

Other inflammatory airways diseases which may be treated with compounds of the invention include pneumoconiosis (an inflammatory, commonly occupational, disease of the lungs occasioned by repeated inhalation of dusts) including for example aluminosis, asbestosis, chalicosis, siderosis, silicosis, tabacosis and byssinosis.

Further inflammatory airways diseases which may be treated with compounds of the invention include adult respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD) in the exacerbation phase thereof and exacerbation of airways hyperactivity consequent to other drug therapy, e.g. aspirin or β-agonist bronchodilator therapy.

In view of their anti-inflammatory activity, particularly in relation to inhibition of eosinophil activation, compounds of the invention are also useful for the treatment of related disorders of the airways, e.g. eosinophilia, hypereosinophilia, eosinophilic pneumonia, parasitic infestation (including tropical eosinophilia), bronchopulmonary

aspergillosis, polyarteritis nodosa, eosinophilic granuloma and eosinophil-related disorders affecting the airways caused by drug- reaction.

Compounds of the invention may also be used in the treatment of allergic inflammatory diseases such as allergic rhinitis.

The compounds of the invention are particularly useful for inhibiting transplant rejection by transplant recipients (e.g., in heart, lung, combined heart-lung, liver, heart, kidney, pancreas, skin or corneal transplants), including both allo- and xeno-graft rejection. The compounds of the invention are also indicated for the prevention of graft-versus-host disease, such as following bone marrow transplantation. The compounds of the invention may be used alone or in combination with known immunosuppressive agents. Such immunosuppressive agents include cyclosporine, tacrolimus, mycophenolic acid (mycophenolate mofetil), brequinar (brequinar sodium)), rapamycin and the like. The dose of these immunosuppressive agents required to achieve an immunosuppressive effect when used in combination may then be reduced, thus reducing the incidence of undesirable side effects associated with the particular known immunosuppressive agent, e.g., nephrotoxicity in the case of cyclosporine and tacrolimus.

The above-cited properties are demonstrable in vitro and in vivo tests using advantageously mammals, e.g. rats, mice, dogs, monkeys, and isolated cells thereof. Said compounds can be applied in vitro in the form of solutions, e.g., preferably aqueous solutions and in vivo either enterally or parenterally, advantageously orally and intravenously. The dosage in vitro may range between about 10^{-5} and 10^{-9} molar concentrations. The dosage in vivo may range, depending on the route of administration, between about 0.1 and 100 mg/kg.

The cell adhesion inhibitory activity of these compounds may be measured by determining the concentration of inhibitor required to block the binding of VLA-4-expressing cells to fibronectin- or CS1-coated plates. In this assay microtiter wells are coated with either fibronectin (containing the CS-1 sequence) or CS-1. If CS-1 is used, it must be conjugated to a carrier protein, such as bovine serum albumin, in order to

bind to the wells. Once the wells are coated, varying concentrations of the test compound are then added together with appropriately labeled, VLA-4-expressing cells. Alternatively, the test compound may be added first and allowed to incubate with the coated wells prior to the addition of the cells. The cells are allowed to incubate in the wells for at least 30 minutes. Following incubation, the wells are emptied and washed. Inhibition of binding is measured by quantitating the fluorescence or radioactivity bound to the plate for each of the various concentrations of test compound, as well as for controls containing no test compound.

VLA-4-expressing cells that may be utilized in this assay include Ramos cells, Jurkat cells, A375 melanoma cells, as well as human peripheral blood lymophocytes (PBLs). The cells used in this assay may be fluorescently or radioactively labeled.

A direct binding assay may also be employed to quantitate the inhibitory activity of the compounds of this invention. In this assay, a VCAM-IgG fusion protein containing the first two immunoglobin domains of VCAM (D1D2) attached above the hinge region of an IgG1 molecule (VCAM 2D-IgG), is conjugated to a marker enzyme, such as alkaline phosphatase (AP). The synthesis of this VCAM-IgG fusion is described in PCT publication WO 90/13300. The conjugation of that fusion to a marker enzyme is achieved by well known crosslinking methods. The VCAM-IgG enzyme conjugate is then placed in the wells of a multi-well filtration plate, such as that contained in the Millipore Multiscreen Assay System (Millipore Corp., Bedford, MA). Varying concentrations of the test inhibitory compound are then added to the wells followed by addition of VLA-4-expressing cells. The cells, compound and VCAM-IgG enzyme conjugate are mixed together and allowed to incubate at room temperature. Following incubation, the wells are vacuum drained, leaving behind the cells and any bound VCAM. Quantitation of bound VCAM is determined by adding an appropriate colorimetric substrate for the enzyme conjugated to VCAM-IgG and determining the amount of reaction cell adhesion inhibitory activity.

In order to assess the VLA-4 inhibitory specificity of the compounds of this invention, assays for other major groups of integrins, i.e., β2 and β3, as well as other β1 integrins,

such as VLA-5, VLA-6 and α4β7 are performed. These assays may be similar to the adhesion inhibition and direct binding assays described above, substituting the appropriate integrin-expressing cell and corresponding ligand. For example, polymorphonuclear cells (PMNs) express β2 integrins on their surface and bind to ICAM. β3 integrins are involved in platelet aggregation and inhibition may be measured in a standard platelet aggregation assay. VLA-5 binds specifically to Arg-Gly-Asp sequences, while VLA-6 binds to laminin.

An in vivo assay which tests the inhibition of contact hypersensitivity in an animal is described in P.L. Chisholm et al., Eur. J. Immunol., vol. 23, pp. 682-688 (1993).

An assay which measures the inhibition of *Ascaris* antigen-induced late phase airway responses and airway hyperresponsiveness in asthmatic sheep is described in W.M. Abraham et al., J. Clin. Invest., vol. 93, pp. 776-87 (1994).

The compounds of the invention may also be tested in the antigen-induced pulmonary eosinophilia assay in the mouse, as described below.

Male B6D2F1/J mice are sensitized by i.p. injection of 0.5 mL alum-precipitated antigen containing 8 μg of ovalbumin (OVA) adsorbed to 2 mg of aluminum hydroxide gel in a saline vehicle. Five days later the mice are given a booster injection with OVA/alum. Control animals are sensitized with alum only. Ten mice are used for each group.

Mice are placed in a 12x14x10 inch plexiglass chamber and exposed to aerosolized OVA (0.5% in saline) for 1 hour at the beginning of the experiment (t = 0), and five hours later.

The antagonists are dissolved in 2% DMSO and 150 mM TRIS, pH 8.8. A solvent control is included for each experiment. Drugs are administered orally 30 minutes prior to OVA exposure, and 6 hour after the first OVA exposure.

The % inhibition is calculated by the formula:

$$1 - \frac{(\#\text{ Eos with drug in OA group} - \#\text{ Eos in no OA group})}{(\#\text{Eos in OA group} - \#\text{ Eos in no OA group})} \times 100\%$$

where:

Eos = average number of eosinophils

OA group = challenged mice

no OA group = unchallenged mice.

Animals are sacrificed by CO_2 asphyxiation 24 hour after the first antigen challenge. The tracheas are exposed and cannulated. The lungs are lavaged with 0.6 mL buffer (Hanks buffered saline with 10 mM Hepes, 0.5% BSA and 10 U/mL heparin). The number of eosinophils in the lavage is assessed by counting the total number of leukocytes and the percentage of eosinophils for each sample.

The immunosuppressive activity can also be determined in further animal models of T-cell mediated immune responses such as experimental allergic encephalomyelitis, Freund's adjuvant or collagen-induced arthritis, and models of graft vs. host reactions.

The antiarthritic activity can be determined in the collagen II induced arthritis in mice model. DBA/1LacJ female mice, 6-8 weeks old, are immunized with 100 μg of chicken type II collagen emulsified in Freund's complete adjuvant (FCA) by injecting at the base of the tail on day 0. On day 17 after collagen injection, the immune response to type II collagen is boosted with a subcutaneous injection of 200 μg LPS solubilized in PBS. Paws are examined on days 17, 21, 24, 28, 31, 35, and 42 and scored for the symptoms of arthritis based on criteria such as inflammation, swelling and ankylosis. Compounds are suspended in corn starch vehicle and administered orally to mice once daily beginning on day 1 and continuing through day 42.

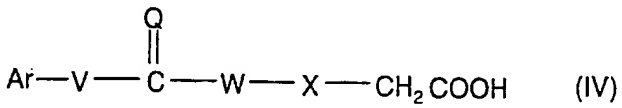
Protection against transplant rejection can be determined in the following mouse tail skin transplantation procedure.

Mice are anesthetized with tribromoethanol i.p. injection according to the method of Papaioannou and Fox (Lab. Animal Science 1993;43:189-92).. The mouse tail skin

transplant rejection model is a modification of that described by Baily and Usama, (Transpl. Bull. 1960;7:424-5). Four skin grafts per mouse are exchanged between strains [C57BL/10-SnJ (H-2K^b) and B10.BR/SgSnJ (H-2K^k)] at surgery (d0). Fitted skin grafts are made with a scalpel, and tail graft sites are protected with tubing for 2 days following surgery. Mouse tails are evaluated at day 7 for missing grafts. Only those graft sites that have patent grafts at day 7 are evaluated at later scoring intervals. Grafts are scored numerically for signs of rejection at ~weekly scoring intervals starting day 14. Dose-group mean graft scores are compared statistically. Drug treatment is considered efficacious if graft scores of compound treated mice are significantly lower than those of vehicle treated mice at days 20/21 and 27/28.

The compounds of the invention can be prepared by adaptation of previously reported synthetic methodology, e.g., in International Application WO 96/22966 (designating the U.S. and which is incorporated herein by reference) as described below and illustrated in the examples.

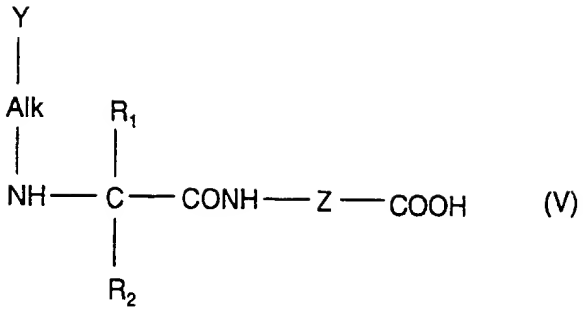
Compounds of formula I are prepared by reacting a compound of formula IV



(IV)

wherein Ar, V, Q, W, X have meaning as defined hereinabove, or a reactive functional derivative thereof, with a compound

of the formula V



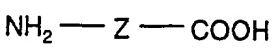
(V)

wherein the carboxyl group is in protected form and wherein Alk, Y, R_1, R_2 and Z have meaning as defined hereinabove, and if required, converting a compound so obtained to another compound of the invention.

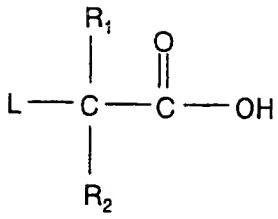
The condensation is carried out according to methodology well known in the art for amide formation, e.g. in the presence of a condensing agent such as 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride and a base, such as diisopropylethylamine, in an inert solvent (such as methylene chloride), preferably at room temperature.

The starting materials of formula IV, such as optionally substituted phenylureidophenylacetic acids, are in turn known in the art or are prepared according to methods known in the art, e.g. by, for example, condensing a p-aminophenylacetic acid ester with the appropriate aryl isocyanate to obtain the corresponding phenylureidophenylacetic acid ester and hydrolyzing the resulting ester.

The starting materials of formula V are in turn prepared by reacting a compound of the formula



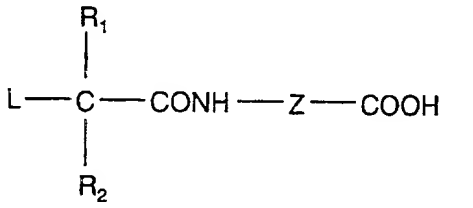
wherein the carboxyl group is in protected form (e.g. as an alkyl ester) and Z has meaning as defined hereinabove, with a compound of the formula



(VII)

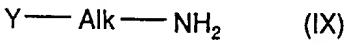
preferably as a reactive functional derivative thereof,
wherein R_1 and R_2 have meaning as defined hereinabove and L is a leaving group, such as halo or (alkyl or aryl)-sulfonyloxy, in the presence of a base, such as triethylamine,

to obtain a compound of the formula VIII



(VIII)

wherein the carboxylic acid is in protected form (e.g. as an alkyl ester), and L, R_1, R_2 and Z have meaning as defined hereinabove, which is in turn reacted with an amine of the formula IX



(IX)

wherein Y and Alk have meaning as defined hereinabove, under conditions well-known in the art, to obtain a starting material of formula V in protected form (e.g. as an alkyl ester). Hydrolysis, e.g. with base, such as aqueous lithium hydroxide, gives a starting material of formula V.

As noted above in the cited processes, such may be carried out while, if necessary, temporarily protecting any interfering reactive group(s), and then liberating the resulting compound of the invention.

In starting compounds and intermediates which are converted to the compounds of the invention in a manner described herein, functional groups present, such as carboxyl, amino and hydroxy groups, are optionally protected by conventional protecting groups that are a common in preparative organic chemistry.

Well-known protecting groups and their introduction are described, for example, in J.F.W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London, New York, T.W. Greene, "Protective Groups in Organic Synthesis", Wiley, New York. For example, a hydroxy group is advantageously protected in the form of a benzyl ether which can be cleaved by catalytic hydrogenation to obtain a hydroxy substituted product.

The above-mentioned reactions are carried out according to standard methods, in the presence or absence of diluent, preferably such as are inert to the reagents and are solvents thereof, of catalysts, condensing or said other agents respectively and/or inert atmospheres, at low temperatures, room temperature or elevated temperatures (preferably at or near the boiling point of the solvents used), and at atmospheric or super-atmospheric pressure. The preferred solvents, catalysts and reaction conditions are set forth in the appended illustrative examples.

The invention also relates to any novel starting materials and processes for their manufacture.

Any mixtures of final products or intermediates obtained can be separated on the basis of the physico-chemical differences of the constituents, in known manner, into the pure final products or intermediates, for example by chromatography, distillation, fractional crystallization, or by formation of a salt if appropriate or possible under the circumstances.

The compounds of the invention or intermediates can also be obtained in the form of their hydrates, or include other solvents used for their crystallization.

Some of the compounds described herein contain one or more asymmetric centers and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms which may be defined in terms of absolute stereochemistry as (R) or (S), or as (D) or (L) for amino acids. The present invention is meant to include all such possible diastereomers as well as their racemic and optically pure forms. Optically active (R) and (S), or (D) and (L), isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended to include both E and Z geometric isomers. Likewise, all tautomeric forms are intended to be included.

The invention further relates to pharmaceutical compositions suitable for enteral, such as oral or rectal, transdermal, intranasal, topical ocular and parenteral administration to mammals including man, which are useful as VLA-4 antagonists and for the treatment of disorders responsive thereto, comprising an effective amount of a pharmacologically active compound of the invention, alone or in combination, with one or more pharmaceutically acceptable carriers.

The compositions include compositions suitable for oral, rectal, topical (including transdermal devices, aerosols, creams, ointments, lotions, and dusting powders), parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation), or nasal administration; the most suitable route in any given case will depend largely on the nature and severity of the condition being treated and on the nature of the active ingredient. The compounds of the invention may be conveniently presented in a unit dosage form prepared by any of the methods well known in the art of pharmacy.

For example, in the treatment of airways diseases, compounds of the invention may be administered orally, for example in tablet form, or by inhalation, for example in aerosol or other atomisable formulations or in dry powder formulations, using an appropriate inhalation device such as those known in the art. For use in the treatment of allergic rhinitis, the compounds of the invention may also be administered intranasally. For the treatment of ocular disorders, the compounds of the invention may also be administered topically e.g. as an eye drop, gel, ointment and the like.

A compound of the invention may be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the nature of the preparation desired for administration, i.e., oral, parenteral, etc. In preparing oral dosage forms, any of the usual pharmaceutical media may be used, such as water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid preparations (e.g., suspensions, elixirs, and solutions); or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, etc. in the case of oral solid preparations such as powders, capsules, and tablets. Solid oral preparations are generally preferred over liquid oral preparations. Because of their ease of administration, tablets and capsules are the preferred oral dosage unit form. If desired, tablets may be coated by standard aqueous or non-aqueous techniques.

In addition to the dosage forms described above, the compounds of the invention may be administered by controlled release means and devices, e.g. a transdermal therapeutic system.

Pharmaceutical compositions of the present invention suitable for oral administration may be prepared as discrete units such as capsules, cachets, or tablets each containing a predetermined amount of the active ingredient in powder or granular form or as a solution or suspension in an aqueous or nonaqueous liquid or in an oil-in-water or water-in-oil emulsion. Such compositions may be prepared by any of the methods known in the art of pharmacy. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers, finely divided solid

carriers, or both and then, if necessary, shaping the product into the desired form. For example, a tablet may be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as powder or granule optionally mixed with a binder, lubricant, inert diluent, or surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent.

Injectable compositions are preferably aqueous isotonic solutions emulsions or suspensions, and suppositories are advantageously prepared from fatty emulsions or suspensions. Said compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In addition, they may also contain other therapeutically valuable substances. Said compositions are prepared according to conventional mixing, granulating or coating methods, respectively, and contain about 0.1 to 75%, preferably about 1 to 50% of the active ingredient.

Suitable formulations for transdermal application include an effective amount of a compound of the invention with carrier. Advantageous carriers include absorbable pharmacologically acceptable solvents to assist passage through the skin of the host. Characteristically, transdermal devices are in the form of a bandage comprising a backing member, optionally a reservoir containing the compound optionally with carriers, optionally a rate controlling barrier to deliver the compound of the skin of the host at a controlled and predetermined rate over a prolonged period of time, and an adhesive layer to secure the device to the skin.

Depending on the indication, the compositions containing a compound of this invention may also comprise an additional agent selected from the group consisting of e.g. cortiocosteroids, bronchodilators, antiasthmatics (mast cell stabilizers), anti-inflammatories, antirheumatics, immunosuppressants, antimetabolites, immunonodulators, antipsoriatics, and antidiabetics. Specific compounds that can be used in combination include

cyclosporine, FK-506, and rapamycin (immunosuppressants); cyclophosphamide and methotrexate (antimetabolites); and interferons (immunomodulators).

The invention further relates to a method of inhibiting VLA-4 activity in mammals and treating or preventing diseases and conditions responsive thereto described herein, e.g. autoimmune disorders, rejection of transplantation, psoriasis or respiratory diseases which comprises administering to a mammal in need thereof an effective amount of a compound of the invention or of a pharmaceutical composition comprising a said compound in combination with one or more pharmaceutically acceptable carriers.

A particular embodiment thereof relates to a method of inhibiting VLA-4 dependent cell adhesion in mammals which comprises administering to a mammal in need thereof a correspondingly effective inhibiting amount of a compound of the invention or of a said compound in combination with one or more pharmaceutically acceptable carriers.

The prophylactic or therapeutic dose of the compounds of the invention will vary with the nature and severity of the condition to be treated and with the particular compound of the invention and its route of administration. In general, the daily dose lies in the range of 0.05 to 50 mg/kg body weight of a mammal, preferably in the range of 0.1 to 10 mg/kg, in single or divided doses. In some cases, it may be necessary to use doses outside these ranges.

A unit dosage for a mammal of about 50 to 70 kg may typically contain between about 1 and 150 mg of the active ingredient.

The following examples are intended to illustrate the invention and are not to be construed as being limitations thereon. Temperatures are given in degrees Centigrade. If not mentioned otherwise, all evaporations are performed under reduced pressure, preferably between about 15 and 100 mm Hg. The structure of final products, intermediates and starting materials is confirmed by standard analytical methods, e.g. microanalysis and/or spectroscopic characteristics (such as MS, IR, NMR and UV).

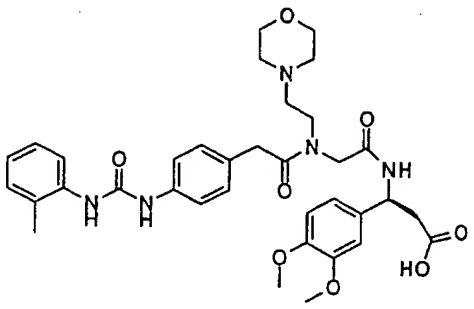
Depending on the chemical nature of the substituent at the asymmetric carbon, an enantiomer is, according to conventional rules of nomenclature, named (R) or (S).

The following abbreviations have the indicated meaning:

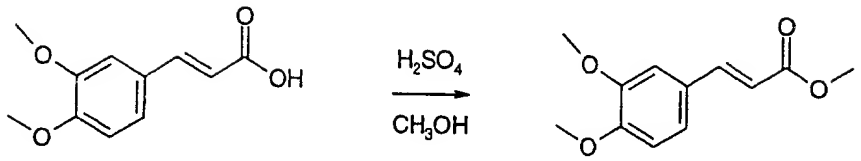
DIC	=	N,N'-diisopropylcarbodiimide
DIEA	=	diisopropylethylamine
DMAP	=	4-dimethylaminopyridine
DMSO	=	dimethyl sulfoxide
EDAC	=	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
HOBT	=	N-hydroxybenzotriazole
HOSu	=	N-hydroxysuccinimide
HPLC	=	high pressure liquid chromatography
MS	=	mass spectroscopy
NMR	=	nuclear magnetic resonance
TEA	=	triethylamine
TLC	=	thin layer chromatography
TRIS	=	tris(hydroxymethyl)aminomethane

Example 1

(S)-β-[[p-(o-Tolylureido)-phenylacetyl-N-(2-morpholinoethyl)-glycyl]amino]-β-(3,4-dimethoxyphenyl)propionic acid



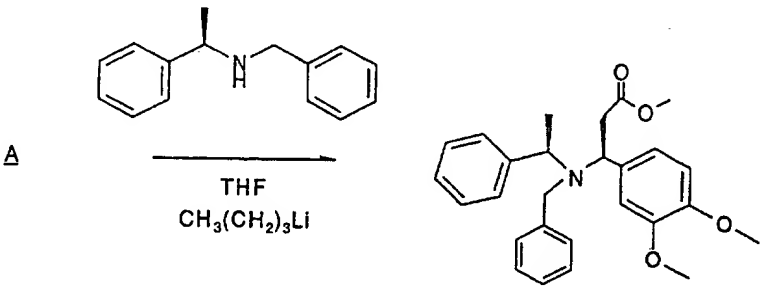
Step 1



A

To 300 mL CH_3OH is added 30 g (144.2 mmol) 3,4-dimethoxycinnamic acid. Four drops H_2SO_4 is added and the mixture refluxed for 4 hours. TLC, using 70/30 ethyl acetate/hexanes, is used to monitor the reaction. The mixture is reduced to dryness and flash chromatographed, using 20% ethyl acetate/80% hexanes, on 350 g silica gel, grade 60, 70-230 mesh, to yield methyl 3,4-dimethoxyphenylcinnamate (Product A).

Step 2

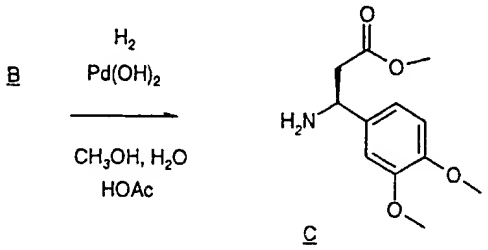


B

To 200 mL THF is added 11.8 g (55.8 mmol) (R)-(+)-N-benzyl-α-methylbenzylamine. The mixture is cooled to 0°C and 34.9 mL (55.8 mmol) n-BuLi (1.6 M in hexanes) added dropwise over 30 min. The mixture is stirred for an 30 additional minutes. The reaction

is cooled to -78°C. Then 6.2 g (27.9 mmol) methyl 3,4-dimethoxycinnamate (A), dissolved in 150 mL THF, is added dropwise over 1 hour. The mixture is stirred for 30 minutes at -78°C and slowly, maintaining the temperature at -78°C, 25 mL saturated NH_4Cl solution is added and the mixture warmed to room temperature, washed with brine, and reduced to dryness. TLC, using 50/50 ethyl acetate/hexanes, is used to monitor the reaction. The mixture is flash chromatographed on 180 g silica gel, Merck, grade 9385 (230-400 mesh, 60A) to yield product B as a thick yellow oil.

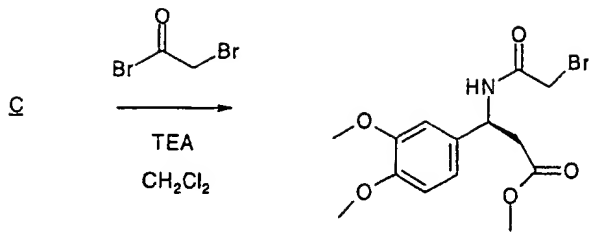
Step 3



C

5.0 g (11.5 mmol) B is added to 250 mL CH_3OH, 25 mL H_2O, and 7.5 mL HOAc. 1 g Pearlman's catalyst ($Pd(OH)_2$) is added. Using a balloon, the mixture is refluxed in an H_2 atmosphere for 16 hours at room temperature. TLC, using 5% CH_3OH/CH_2Cl_2, is used to monitor the reaction. The mixture is filtered through celite, washed with CH_3OH, and reduced to dryness. To the dry product is added CH_2Cl_2 and the solution is washed with brine and made basic with saturated $NaHCO_3$. The mixture is reduced to dryness and flash chromatographed using 150 g silica gel (230-400 mesh), and 1 to 4% CH_3OH/CH_2Cl_2 as eluant to yield methyl β-amino-β-(3,4-dimethoxy-phenyl) propionate (product C) as a yellow oil.

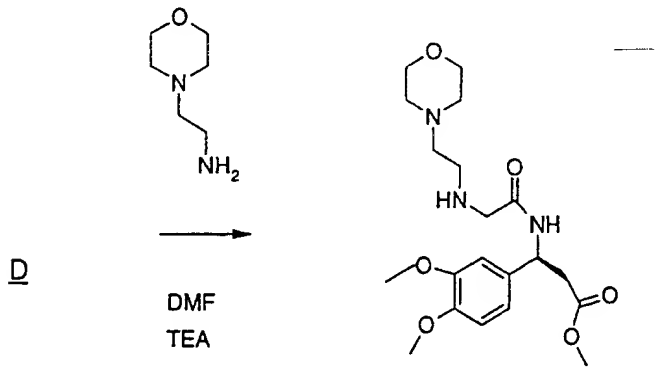
Step 4



D

To 9 mL CH_2Cl_2 is added 0.2 g (0.8 mmol) C and 0.13 mL (0.9 mmol) triethylamine. The mixture is stirred 10 minutes and cooled to 0°C. 0.08 mL (0.9 mmol) Bromoacetyl bromide in 1 mL CH_2Cl_2 is added dropwise over 15 minutes. The mixture is stirred over 3 hours allowing the mixture to reach room temperature. TLC, using 50% ethyl acetate/50% hexanes, is used to monitor the reaction. The mixture is reduced to dryness and flash chromatographed using 30 g silica gel, Merck, grade 9385 (230-400 mesh, 60 A) using 25% ethyl acetate/75% hexanes as eluant, to yield methyl β-(3,4-dimethyoxyphenyl)-β-(bromoacetylamino) propionate (product D) as a thick yellow oil.

Step 5

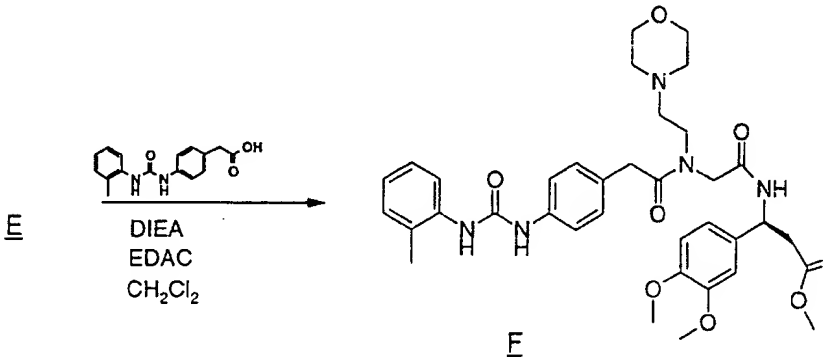


E

To 10 ml DMF are added 0.36g (1mmol) D and 0.35g (2.5 mmol) of 4-(2-aminoethyl) morpholine. At room temperature 0.23 mL TEA is added. The mixture is stirred 16 hours at room temperature. TLC, using 10% CH_3OH in CH_2Cl_2, is used to monitor the reaction. The mixture is reduced to dryness and flash chromatographed using 12g

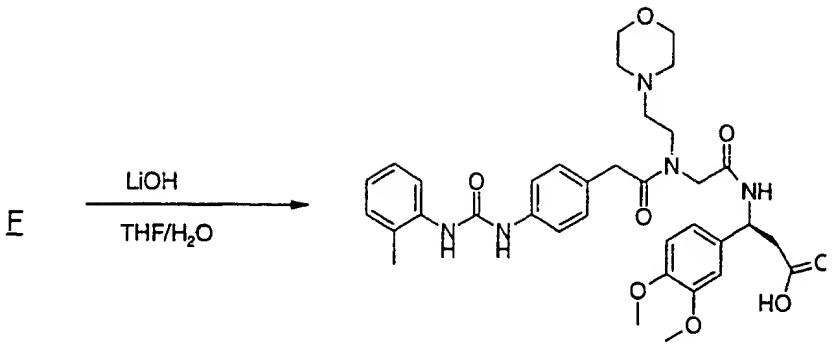
silica gel, starting with 2% and gradually increasing to 4% CH_3OH in CH_2Cl_2, to yield methyl β-[(N-morpholinoethylglycyl)amino]-β-(3,4-dimethoxyphenyl) propionate (product E) as a yellow oil.

Step 6



To 40 ml CH_2Cl_2 is added 1.2 g(3.0 mmol) E. Then 1.0 g (3.5 mmol) of N-(o-tolyl)-N'-(phenyl-4'-acetic acid) urea, (only partially soluble) and 0.57 mL (4.1 mmol) of diisopropylethylamine (DIEA) are added and the mixture stirred 15 minutes to give a clear yellow solution. Then 0.70 g (3.5 mmol) 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDAC) is added and the mixture stirred 3 hours. TLC, using 10% CH_3OH in CH_2Cl_2, is used to monitor the reaction. The mixture is reduced to dryness, flash chromatographed eluting with 2% CH_3OH in CH_2Cl_2, to yield methyl (S)-β-[[p-(o-tolylureido)phenylacetyl-N-(2-morpholinoethyl)-glycyl]amino]-β-(3,4-dimethoxyphenyl) propionate (product F) as a white foam.

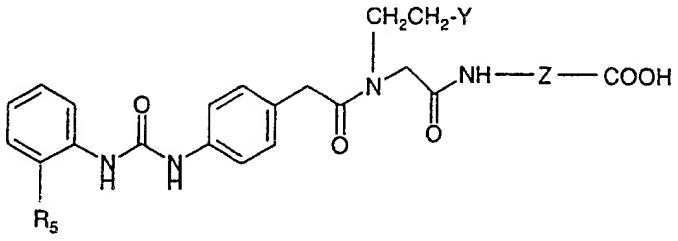
Step 7

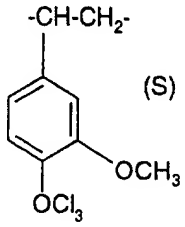
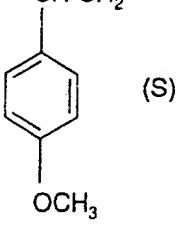
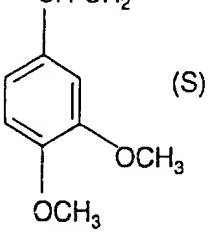


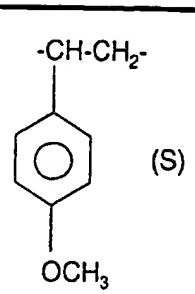
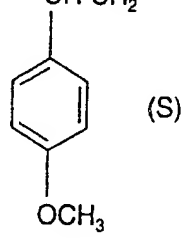
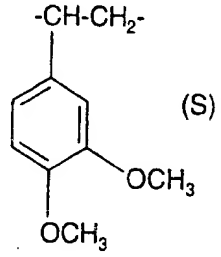
To 45 mL THF and 13 mL H_2O is added 1.4 g (2.1 mmol) of product F. 0.132 g(3.1 mmol) of LiOH dissolved in 2 mL H_2O is added dropwise over 5 minutes and the mixture stirred for 2 hours at room temperature. TLC, using 10% CH_3OH in CH_2Cl_2, is used to monitor the reaction. The mixture is reduced to dryness. H_2O is added to the solid, the mixture is adjusted to pH 2, neutralized to pH = 7, extracted with THF, dried and evaporated to dryness to give the title compound as white solid; mp: 120-125°C (dec); $[\alpha]$ -16.074° in DMSO (10 mg/mL).

Example 2

Similarly to the procedure of Example 1 are prepared the following compounds (as the indicated enantiomers if in optically active form).

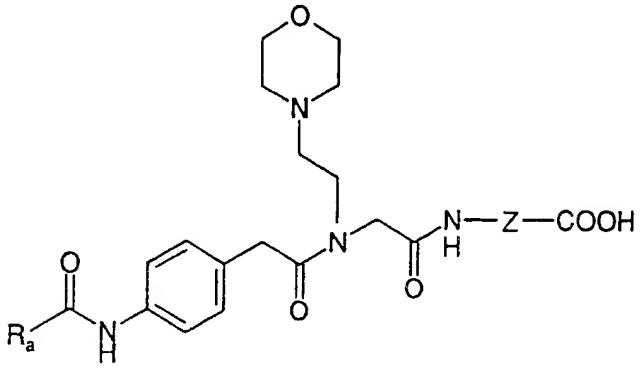


Compound	R_5	Y	Z/Enantiomer	m.p. (°C)
(a)	CH_3	dimethylamino		
(b)	CH_3	morpholino	$-CH_2-C(CH_3)_2-$	100-105
(c)	CH_3	morpholino		149-153
(d)	OCH_3	morpholino		132-135
(e)	CH_3	morpholino	$-CH_2CH_2-$	125-128
(f)	CH_3	morpholino	$-CH(CH_3)-CH_2-$ (S)	104-106

(g)	OCH_3	morpholino		139-143
(h)	F	morpholino		136-138
(i)	F	morpholino		140-143
(j)	F	morpholino	$-CH_2CH_2-$	121-125
(k)	F	morpholino	$-CH(CH_3)CH_2-$ (S)	108-112
(l)	OCH_3	morpholino	$-CH_2CH_2-$	129-134
(m)	CH_3	morpholino	$-(CH_2)_2-S-CH_2-$	116-119
(n)	CH_3	morpholino	$-(CH_2)_2-SO-CH_2-$	136-140
(o)	CH_3	morpholino	$-(CH_2)_4-$	130-134
(p)	CH_3	morpholino	$-CH_2-CH(CH_3)-$	131-135

Example 3

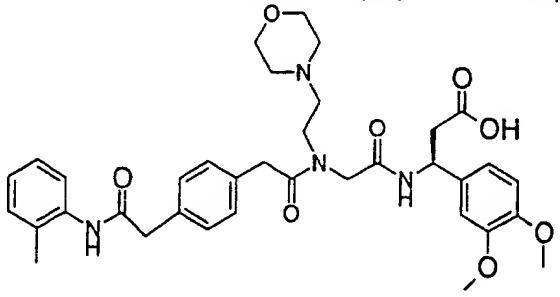
Similarly to procedures in Example 1 are prepared the following compounds:



Compound	R_a	Z	m.p. (°C)
(a)	3-indolyl	-CH(CH$_3$)-CH$_2$- (S)	122-136
(b)	3-indolyl	-(CH$_2$)$_4$-	95-98
(c)	o-methyl-benzyl	-CH(CH$_3$)CH$_2$- (S)	130-133

Example 4

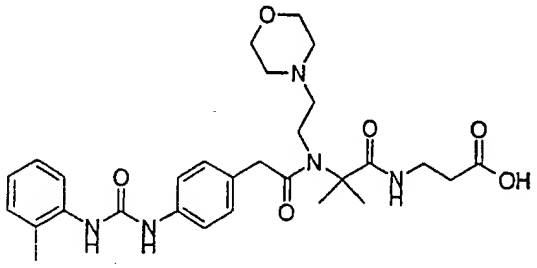
Similarly to procedures in Example 1 is prepared the compound of the formula



having a melting point of 145-151°C dec.

Example 5

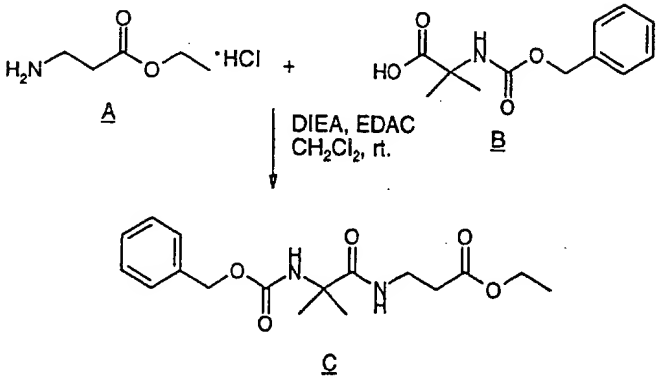
Similarly to procedures in Example 1 is prepared the compound of the formula



having a melting point of 148-151°C.

The starting material is prepared as follows:

Step 1

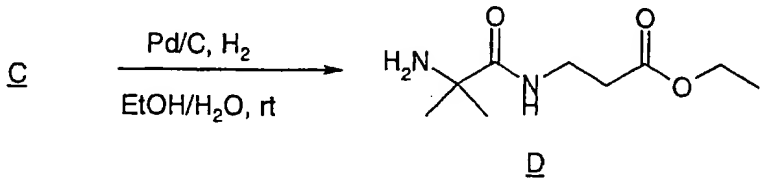


C

To 30 mL CH_2Cl_2 is added 0.66 g (4.3 mmol) of A. At room temperature, 1.0 g (4.2 mmol) of B, and 1.5 mL (8.6 mmol) DIEA are added, and the mixture is stirred 15 minutes to give a clear yellow solution. 0.84 g (4.3 mmol) EDAC is added and the

mixture stirred 3 hours. MS is used to monitor the reaction. The mixture is reduced to dryness, flash chromatographed (2% CH_3OH in CH_2Cl_2) to yield product C as a pale yellow oil.

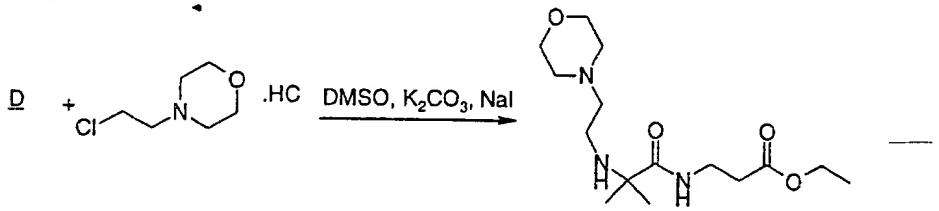
Step 2



D

To 50 mL EtOH/3 mL H_2O is added 0.84 g (2.5 mmol) of C. 0.07 g Pd/C (as catalyst) is added to the mixture. The mixture is stirred under a hydrogen balloon for 2 hours. MS is used to monitor the reaction. The mixture is filtered, washed with EtOH, and dried to give product D as a pale yellow oil.

Step 3



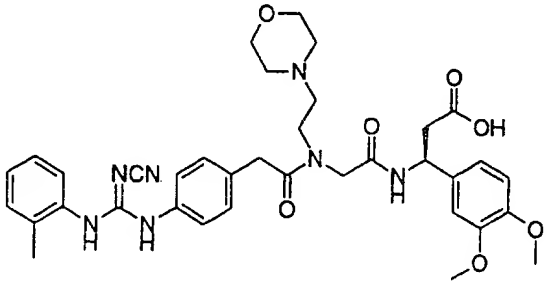
E

To 5 mL DMSO are added 0.1 (0.5 mmol) D, 0.046 g (0.25 mmol) 4-(2-chloroethyl)morpholine hydrochloride, 0.10 g (0.75 mmol) potassium carbonate and 0.04 g sodium iodide(as catalyst). The mixture is stirred for 24 hours. MS is used to monitor the reaction. The mixture is reduced to dryness, flash chromatographed (2% CH_3OH in CH_2Cl_2) to give product E as a pale yellow oil.

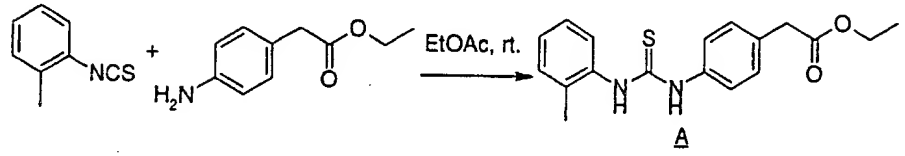
Conversion of product E to the title compound is carried out similarly to final steps in Example 1.

Example 6

(S)-β-[[p-(N'-o-tolylcyanoguanidino) phenylacetyl-N-(2-morpholinoethyl) glycyl] amino]-β-(3,4-dimethoxyphenyl)propionic acid



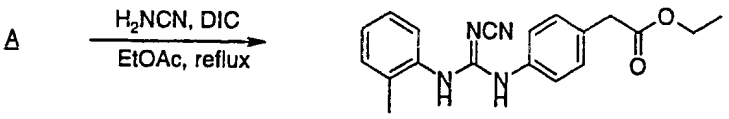
Step 1



A

To 25 mL EtOAc are added 1 g (6.7 mmol) o-tolylsothiocyanate and 1.32 g (6.7 mmol) ethyl 4-aminophenyl acetate. The mixture is stirred for 12 hrs. at room temp. TLC, using as solvent 50% hexanes in EtOAc, is used to monitor the reaction. The mixture is reduced to dryness and flash chromatographed on 45 g silica gel, eluting with CH_2Cl_2 to give ethyl 4-N- (o-tolylamino thiocarbonyl)aminophenylacetate (product A) as a yellow solid.

Step 2

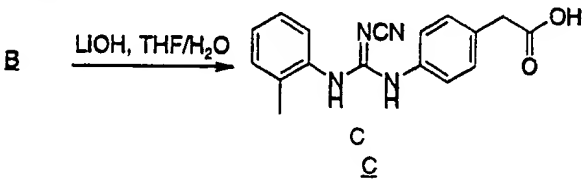


B

To 50 mL EtOAc are added 1.6 g(4.87 mmol) A, 0.31 g (7.31 mmol) cyanamide and 0.92 g (7.31 mmol) N,N'-diisopropylcarbodiimide (DIC). The mixture is heated to reflux and stirred for 60 hrs. MS is used to monitor the reaction. The mixture is reduced to

dryness and flash chromatographed on 30 g silic gel, elutingwith CH_2Cl_2 to give ethyl 4-[p-(N'-o-tolylcyanoguanidino)]phenylacetate (product B) as a thick oil.

Step 3



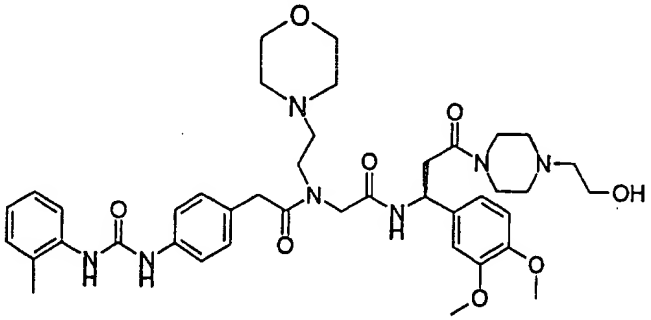
C

To 15 mL THF is added 356 mg (1.06 mmol) B . 92 mg (2.2 mmol) LiOH dissolved in 3.5 mL H_2O is added dropwise and the mixture stirred 4 hrs. at room temperature. MS is used to monitor the reaction. The mixture is reduced to dryness. Then H_2O is added to the residue, the solution is adjusted pH to 2 with 1 N HCl, neutralized to pH=7 with saturated Na_2CO_3 and extracted with THF. The extract is dried and solvent is removed to give 4-[p-(N'-o-tolylcyanoguanidino)] phenylacetic acid (product C) as a white solid.

Product C is condensed with product D of Example 1, and the resulting ester is hydrolyzed (similarly to steps 6 and 7 of Example 1) to the title product which is purified by flash chromatography eluting with 10% CH_3OH in CH_2Cl_2; m.p. 102-106° dec.

Example 7

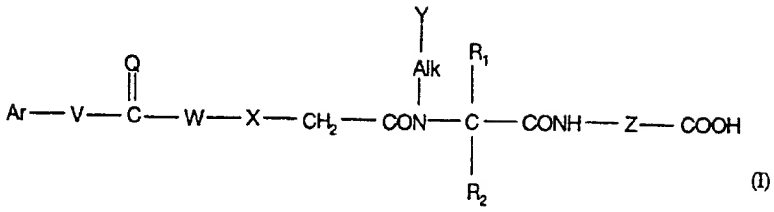
(S)-1-{β-[[p-(o-tolylureido)phenylacetyl-N-(2-morpholinoethyl)-glycyl]amino]-β-(3,4-dimethoxyphenyl)-propionyl}-4-hydroxyethylpiperazine.



To 5 mL CH_2Cl_2 is added 0.16 mmol of the compound of Example 1; 0.022 mL(0.18 mmol) 1-(2-hydroxyethyl)piperazine and 22.5 mg(0.18 mmol) DMAP are added to the mixture. The mixture is stirred at room temperature for 5 minutes and 34 mg (0.18 mmol) EDAC is added. TLC is used to monitor the reaction. After completion of the reaction, the mixture is reduced to dryness. The product is purified by HPLC to give title compound.

WHAT IS CLAIMED IS:

1. A compound of the formula



(I)

wherein

Ar is carbocyclic or heterocyclic aryl, or biaryl;

Q is O, S or N-C≡N;

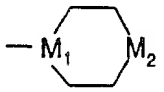
X is arylene;

V is NH, O, NHOH, CH_2 or a direct bond;

W is NH, O, NHOH CH_2 or a direct bond;

Alk is C_2-C_7-alkylene or C_2-C_7-alkylene interrupted by O, S, SO, SO_2 or NR_3;

Y is amino, acylamino, mono- or di- (lower alkyl, aryl or aralkyl)-amino, pyrrolidino, perhydroazepino or a group of the formula



in which M_1 is N; and M_2 is CH_2, O, NR_3, S, SO or SO_2;

R_1, R_2 and R_3 are independently hydrogen, lower alkyl, lower alkenyl, cycloalkyl, aryl, cycloalkyl-lower alkyl, aryl-lower alkyl or aryl-lower alkenyl;

Z is lower alkylene or lower alkylene substituted by one or more of lower alkyl, lower alkenyl, cycloalkyl, aryl, cycloalkyl-lower alkyl, aryl-lower alkyl or aryl-lower alkenyl; or Z is lower alkylene interrupted by O, S, SO, SO_2 or NR_3;

a pharmaceutically acceptable ester or amide thereof; or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 wherein V and W are NH or NHOH.

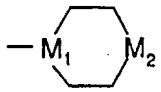
3. A compound of claim 1 wherein V is CH_2 and W is NH.

4. A compound of claim 1 wherein V is a direct bond and W is NH.

5. A compound of claim 1 wherein V is NH and W is CH_2.

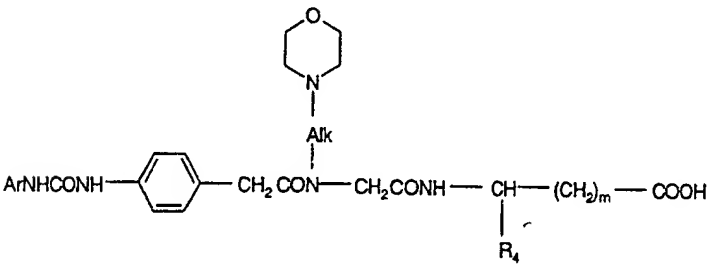
6. A compound of claim 1 wherein Q is O.

7. A compound of claim 1 wherein V and W are NH; Q is O; X is phenylene; Ar is carbocyclic or heterocyclic aryl; Alk is C_2-C_4-alkylene; R_2, R_2 and R_3 are hydrogen or lower alkyl; Y is a group of the formula



in which M_1 is N and M_2 is CH_2, O or S; Z is C_2-C_5- straight chain alkylene optionally substituted by lower alkyl, lower alkenyl, carbocyclic aryl or heterocyclic aryl; or Z is C_2-C_5-straight chain alkylene interrupted by O, S, SO or SO_2; a pharmaceutically acceptable ester or amide thereof; or a pharmaceutically acceptable salt thereof.

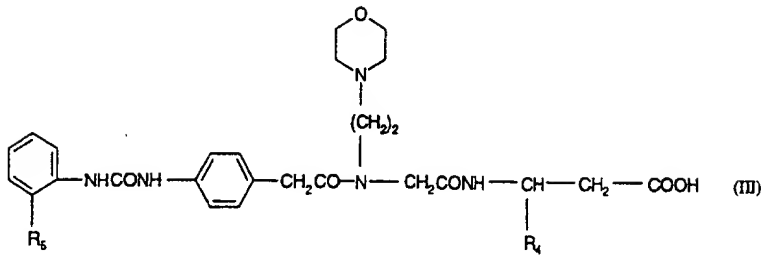
8. A compound of claim 1 of the formula



(II)

wherein Ar is monocarbocyclic aryl; Alk is C_2-C_4-alkylene;
R_4 is lower alkyl, lower alkenyl, or monocarbocyclic aryl; m is 1 or 2; a pharmaceutically acceptable ester thereof; or a pharmaceutically acceptable salt thereof.

9. A compound of claim 8 of the formula



(III)

wherein R_4 is phenyl or phenyl substituted by one to three of C_1-C_4-alkoxy, chloro, fluoro or C_1-C_4-alkyl; and R_5 is C_1-C_4-alkoxy, chloro, fluoro, or C_1-C_4-alkyl; a pharmaceutically acceptable ester thereof; or a pharmaceutically acceptable salt thereof.

10. A compound of claim 9 wherein R_5 is methyl, fluoro or methoxy.

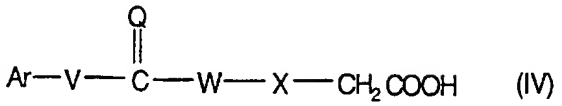
11. A pharmaceutical composition comprising an effective VLA-4 inhibiting amount of a compound of claim 1 in combination with one or more pharmaceutically acceptable carriers.

12. A method of inhibiting VLA-4 activity in mammals which comprises administering to a mammal in need thereof an effective VLA-4 inhibiting amount of a compound of claim 1.

13. A method of treating or preventing VLA-4 dependent conditions in mammals which comprises administering to a mammal in need thereof in effective VLA-4 inhibiting amount of a compound of claim 1.

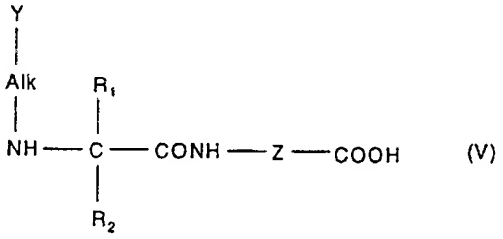
14. A method of treating transplantation rejection, arthritis or respiratory disorders in mammals which comprises administering to a mammal in need thereof a correspondingly effective amount of a compound of claim 1.

15. A method of preparing a compound of claim 1 which comprises reacting a compound of formula IV



(IV)

wherein Ar, V, Q, W, X have meaning as defined hereinabove, or a reactive functional derivative thereof, with a compound of the formula V

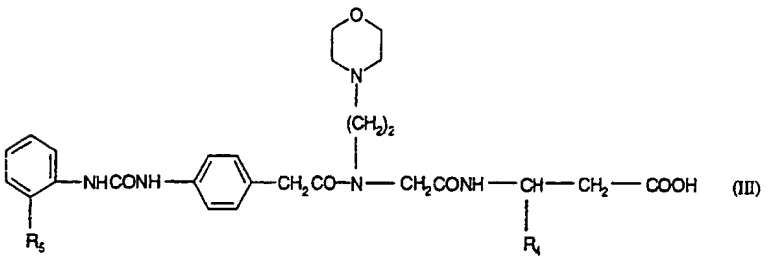


(V)

wherein the carboxyl group is in protected form and wherein Alk, Y, R_1, R_2 and Z have meaning as defined hereinabove, and if required converting a compound so obtained to another compound of claim 1.

16. Use of a compound of claim 1 in the preparation of a pharmaceutical composition for the treatment and/or prevention of VLA-4 dependent conditions in mammals.

17. Use of claim 16, wherein said compound is of the formula (III)



(III)

wherein R_4 is phenyl or phenyl substituted by one to three of C_1-C_4-alkoxy, chloro, fluoro or C_1-C_4-alkyl; and R_5 is C_1-C_4-alkoxy, chloro, fluoro, or C_1-C_4-alkyl; a pharmaceutically acceptable ester thereof; or a pharmaceutically acceptable salt thereof.

18. Use of claim 16, wherein said pharmaceutical composition is adapted for ophthalmic use.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 June 2001 (14.06.2001)

PCT

(10) International Publication Number
WO 01/42192 A3

(51) International Patent Classification[7]: C07C 275/38, C07D 295/13, A61K 31/5375, 31/17, A61P 27/02

(21) International Application Number: PCT/EP00/12226

(22) International Filing Date: 5 December 2000 (05.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/455,670 7 December 1999 (07.12.1999) US

(71) Applicant *(for all designated States except AT, US)*: NOVARTIS AG [CH/CH]; Lichtstrasse 35, CH-4056 Basel (CH).

(71) Applicant *(for AT only)*: NOVARTIS-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H [AT/AT]; Brunner Strasse 59, A-1230 Vienna (AT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: WATTANASIN, Sompong [TH/US]; 11 Divito Trail, Hopatcong, NJ 07832 (US). WEIDMANN, Beat [CH/US]; 20 Van Doren Avenue, Chatham, NJ 07928 (US).

(74) Agent: BECKER, Konrad; Novartis AG, Corporate Intellectual Property, Patent & Trademark Department, CH-4002 Basel (CH).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

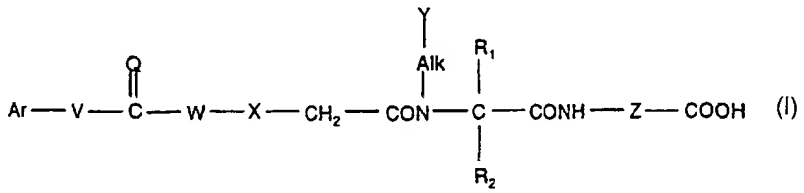
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

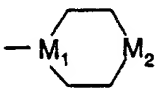
(88) Date of publication of the international search report:
10 January 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: VLA-4 INTEGRIN ANTAGONISTS



(I)



(II)

(57) Abstract: Compounds of formula (I) wherein Ar is carbocyclic or heterocyclic aryl, or biaryl; Q is O, S or N-C≡N; X is arylene; V is NH, O, NHOH, CH_2 or a direct bond; W is NH, O, NHOH, CH_2 or a direct bond; Alk is C_2-C_7-alkylene or C_2-C_7-alkylene interrupted by O, S, SO, SO_2 or NR_3; Y is amino, acylamino, mono- or di- (lower alkyl, aryl or aralkyl)-amino, pyrrolidino, perhydroazepino or a group of the formula (II) in which M_1 is N; and M_2 is CH_2, O, NR_3, S, SO or SO_2; R_1, R_2 and R_3 are independently hydrogen, lower alkyl, lower alkenyl, cycloalkyl, aryl, cycloalkyl-lower alkyl, aryl-lower alkyl or aryl-lower alkenyl; Z is lower alkylene or lower alkylene substituted by one or more of lower alkyl, lower alkenyl, cycloalkyl, aryl, cycloalkyl-lower alkyl, aryl-lower alkyl or aryl-lower alkenyl; or Z is lower alkylene interrupted by O, S, SO, SO_2 or NR_3; pharmaceutically acceptable esters and amides thereof; and pharmaceutically acceptable salts thereof, which are useful as VLA-4 integrin antagonists.

WO 01/42192 A3

INTERNATIONAL SEARCH REPORT

Internat' l Application No
PCT/EP 00/12226

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C275/38 C07D295/13 A61K31/5375 A61K31/17 A61P27/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 37605 A (NOVARTIS ERFIND VERWALT GMBH ;NOVARTIS AG (CH); VON MATT PETER JOS) 29 July 1999 (1999-07-29) page 1 page 6, paragraph 2 page 27, steps 2 and 3 page 28, step 4 page 30, step 4 page 38, step 6 claims ---	1-18
X	WO 98 04247 A (ZHENG ZHONGLI ;ADAMS STEVEN P (US); BIOGEN INC (US); ENSINGER CARO) 5 February 1998 (1998-02-05) page 1, line 1 - line 12 page 27, top structure page 49, compound A --- -/--	1-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 May 2001	06/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	O'Sullivan, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat ' Application No
PCT/EP 00/12226

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 936 065 A (ARRHENIUS THOMAS S ET AL) 10 August 1999 (1999-08-10) abstract table 1	1-18

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

l. mation on patent family members

Internat l Application No
PCT/EP 00/12226

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9937605 A	29-07-1999	AU 2829299 A	09-08-1999
		BR 9907733 A	17-10-2000
		EP 1049665 A	08-11-2000
		NO 20003694 A	20-09-2000
WO 9804247 A	05-02-1998	AU 3738597 A	20-02-1998
		AU 3738697 A	20-02-1998
		BG 103193 A	30-09-1999
		CN 1230110 A	29-09-1999
		CZ 9900232 A	16-06-1999
		EP 0914605 A	12-05-1999
		EP 0917462 A	26-05-1999
		JP 2000516596 T	12-12-2000
		NO 990338 A	25-03-1999
		PL 331332 A	05-07-1999
		SK 8199 A	10-04-2000
		TR 9900781 T	21-07-1999
		WO 9804913 A	05-02-1998
US 5936065 A	10-08-1999	CA 2177840 A	15-06-1995
		CN 1142832 A	12-02-1997
		JP 9509146 T	16-09-1997
		NZ 277206 A	24-11-1997
		US 5821231 A	13-10-1998
		US 5770573 A	23-06-1998
		US 6117840 A	12-09-2000
		AU 693143 B	25-06-1998
		AU 1215695 A	27-06-1995
		EP 0737204 A	16-10-1996
		WO 9515973 A	15-06-1995
		US 5688913 A	18-11-1997
		US 6103870 A	15-08-2000